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Abscisic Acid Elicits the Water-Stress Response in Root Hairs of *Arabidopsis thaliana*[1]

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ABSTRACT

Water stress has been shown to cause root hairs to become short and bulbous. Because abscisic acid (ABA) mediates a variety of water-stress responses, we investigated the response of *Arabidopsis thaliana* root hairs to ABA. When wild-type root hairs were treated with ABA, they exhibited the water-stress response. The *Arabidopsis* mutants *abi1* and *abi2*, which are insensitive to ABA at the seedling stage, did not display the root hair response. These data suggest that ABA may mediate the response of root hairs to water stress. The drought response of root hairs resulting in an inhibition of tip growth will provide an easy screen to select mutations that are insensitive to ABA and/or involved in tip growth.

ABA mediates the drought response in plants by eliciting stomatal closure via a well-documented mechanism (5). Less is known about the effect of ABA on water-stressed roots. Drought stress commonly inhibits root growth (13). However, plants have been known to increase their root to shoot ratio under water-limiting conditions (13). ABA is believed to play a critical role in these responses. Exogenously applied ABA at low concentrations tends to elicit an increase in the root to shoot ratio by preferentially inhibiting shoot growth (2, 11, 13, 14), whereas at high concentrations, it generally inhibits root growth (12, 13); these differences depend on the time and method of ABA application. It is well known that drought stress elicits the accumulation of ABA in roots, particularly at the tips (12, 14). The ABA may, in turn, stimulate ion and sugar accumulation in the root (7, 15, 16), which could affect root turgor. This change in osmotic potential is sometimes accompanied by a reduction in the size of the root meristematic region, along with a decrease in the addition of new cells to the root (12, 13). The causality of this association has not been demonstrated, however. In fact, it has been suggested that the ABA-related change in turgor does not control root growth (6).

To help clarify the role of ABA in the drought response, it seemed worthwhile to examine the effect of water stress on root hairs. These single-cell, highly sensitive structures are known to become abnormally short and swollen under water-limiting conditions (9, 18), especially at the tips (19), and they sometimes seem to disappear under severe water stress (17). The role of ABA in these responses has not been reported. In this paper, we describe the effects of exogenously applied ABA on root hairs of *Arabidopsis thaliana*. The availability of ABA-insensitive mutants allowed us to explore the specificity of the ABA response.

MATERIALS AND METHODS

Seeds of *Arabidopsis thaliana* L. Heynh, race Landsberg *erecta*, including wild type and mutants *abi1*, 2, and 3 were surface sterilized for 5 min in 5% hypochlorite, 0.02% (v/v) Tween 20 (Sigma), followed by five rinses in sterile H_2O. The seeds were sown onto 100- × 20-mm Petri dishes containing 10 mL of 0.2× Murashige and Skoog's medium (10) lacking sucrose, supplemented with 5% (w/v) Mes, pH 5.7, and solidified with 1% Phytagel (Sigma). The plates were sealed with filter tape (Carolina Biologicals, Burlington, NC) and incubated at 4°C for 1 to 3 d, followed by a 5-d incubation, on edge, at 24 ± 1°C, in 16-h light (30 μmol m^{-2} s^{-1}) and 8-h dark photoperiods.

(±)ABA (Sigma) was dissolved in a small amount of ethanol and diluted to 400 μM with sterile H_2O. One milliliter of the solution was applied evenly across the plates. All concentrations are given for the 1-mL treatment solution, but the final concentration was diluted by the 10 mL of solidified media. Plates were incubated horizontally for 2 h to allow the ABA solution to diffuse into the medium, followed by a 2-d incubation, on edge, as described before. Root hairs were counted in the first 2 mm of the zone of maturation of each root. The zone of maturation is defined as the section near the root tip where root hairs first become apparent. Ten to 20 similarly sized roots were scored per treatment, as indicated in the figure legends.

RESULTS AND DISCUSSION

ABA had a striking effect on the appearance of new root hairs (Fig. 1). The new root hairs that were initiated were short and swollen, and root hairs present before the application of the hormone became thin walled, fragile, and difficult to see. These results are similar to those seen in response to drought stress of *Trifolium subterraneum* (18) and *Sinapsis alba* (17). In addition, ABA was found to moderately decrease primary root growth, whereas it had no significant effect on the number of lateral roots initiated (not shown). The similarity between the responses of roots to exogenously applied ABA and to drought stress is not surprising. Water

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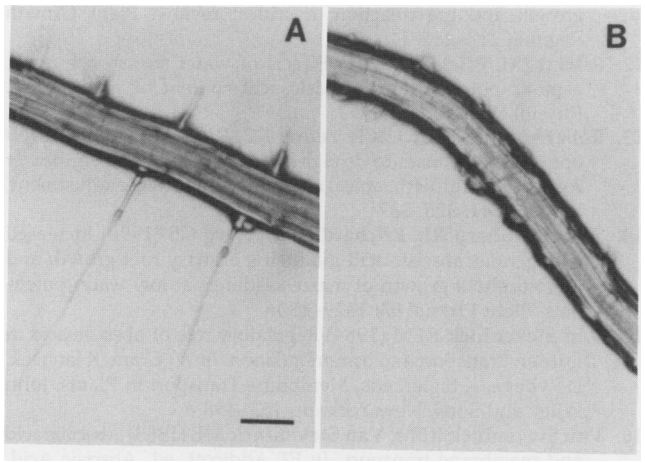

Figure 1. Effect of ABA on wild-type root hairs. A, Control; B, ABA treatment. Scale bar represents 100 μm.

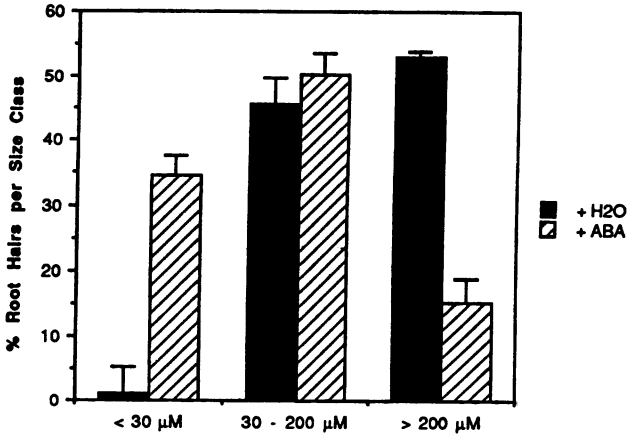

Figure 3. Effect of ABA on *abi* mutants. Root hairs >200 μm in length were counted in a total of 10 roots per treatment, as described in "Materials and Methods." Values are means ± SE.

stress increases endogenous levels of ABA in the roots of a wide variety of plants, including wheat (1), soybean (2), maize (14), *Commelina* (20), and peas (20) and quite possibly *Arabidopsis* as well. Thus, the exogenously applied ABA may serve to simulate the drought response. Indeed, the root hair effect was also elicited by the acetylene acetyl ABA analog LAB 173711. The application of a 100 μM solution of the analog elicited nearly the same response as did the application of 400 μM ABA. This may reflect the relative stability of the analog compared with ABA (4). Neither GA_3, IAA, nor the cytokinin 6-(γ,γ-dimethylallylamino)-purine altered the root hairs in this fashion (not shown).

ABA did not affect the total number of root hairs, but it significantly altered the distribution of root hair size classes (Fig. 2). In both the ABA-treated roots and the H_2O-treated controls, the average number of root hairs contained in 20 2-mm root sections was approximately 38. In the controls, more than half of these root hairs were more than 200 μm in length, and a very small fraction was less than 30 μm long. In contrast, only about 15% of the ABA-treated roots were more than 200 μm long, whereas nearly 35% were shorter than 30 μm. An index of root hairs greater than 200 μm was used in the remainder of the study to monitor the activity of ABA. The number of root hairs greater than 200 μm was directly proportional to the concentration of ABA used. The occurrence of such root hairs was reduced by 45% using 10 μM ABA, whereas the application of 50 and 100 μM resulted in a 66 and 75% reduction in the occurrence of these root hairs, respectively. Application of 500 μM ABA led to a virtual elimination of these long root hairs.

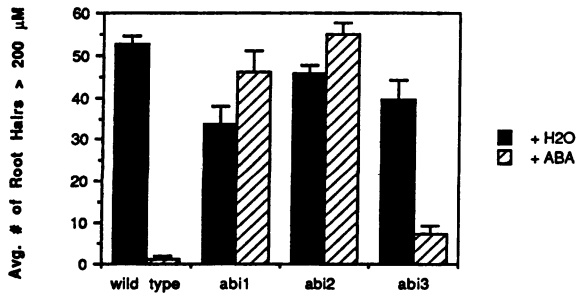

Figure 2. Effect of ABA on the distribution of wild-type root hair size classes. Twenty root sections were counted per treatment, as described in "Materials and Methods." There was an average of approximately 38 root hairs in both control and ABA-treated root sections. Values are means ± SE.

To probe further the specificity of this response, the effect of ABA on ABA-insensitive (*abi*) mutants of *Arabidopsis* (8) was examined (Fig. 3). There was no significant difference in the number of root hairs longer than 200 μm between the control and ABA treatments for *abi1* and *abi2* (*t* test, $P > 0.05$), whereas *abi3* displayed a response similar to that of the wild type. Thus, *abi1* and *abi2* are insensitive to ABA in the root hair response, whereas *abi3* is sensitive. These results are entirely consistent with those described previously (3). The *abi1* and *abi2* alleles were found to function primarily during vegetative growth, i.e. in seedling growth and stomatal function (although they do display limited insensitivity early in seed development). These mutants have a wilted appearance under normal growth conditions because of the insensitivity of their stomates to ABA. The *abi3* allele was determined to function during seed development, particularly in seed storage reserve accumulation. The insensitivity of *abi3* is, therefore, limited to seed development, leading to a normal root hair response in vegetative tissue. The response described in this paper could be utilized to screen for mutants with ABA insensitivity only during vegetative growth and perhaps with altered processes related to tip growth.

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